A HISTOLOGICAL STUDY OF THE OVIDUCT OF THE IMMATURE, MATURE AND PREGNANT BOVINE

by

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INTRODUCTION

Most of the knowledge on the minute structure of the bovine oviduct was based on descriptions of the human and other mammalian oviducts. Beaver (5) was the only person who, as far back as 1922, when working with the complicated problem of finding the etiology of sterility in cattle, made an attempt to understand the histology of the bovine oviducts. He noted the three layers of the oviducts and observed that the tunica muscularis consisted of a strong inner circular layer of smooth muscle fibers with a few external longitudinal fibers. No standard textbook of Histology contained a detailed description of the minute structure of the bovine oviducts. Trautman and Fiebiger (29), in a very brief description covering the oviducts in general of all domestic animals, stated that the columnar epithelium was partly pseudo-stratified in ruminants and swine and that the mucosal folds were less pronounced in ruminants compared to the mare and the sow.

Fertilization normally takes place in the fallopian tubes and so the tube structure is of considerable importance. Veterinarians were interested especially in the biological process and information that gave a clear conception of the structure and the physiological phenomena which furnished a foundation for diagnosis and for a better understanding of and treatment of abnormal conditions. A detailed histological study of normal bovine oviducts will not only help to understand clearly the physiology of reproduction but also in evaluating the pathological processes in the oviduct. Hence the object of this study was to record the normal

microscopic structure of the bovine oviducts during different stages in the life of the animal, namely mature, immature and pregnant, and forms a basic contribution to our knowledge of the area.

REVIEW OF LITERATURE

The work of Heape (16) in 1901 on the phases of sexual season in mammals and its related phenomenon focused, for the first time, the attention of many workers upon the study of the reproductive physiology of many species, including cattle. Studies on the female genital system of cattle, especially in relation to the estrous cycle, has been the subject of many investigators. However, the earlier workers completely neglected the study of the oviducts as they took for granted that the changes occurring in them were insignificant. This could be seen clearly in the work of Hammond (15) who dealt at considerable length on the anatomical and histological changes in the ovaries, uterus, cervix and vagina in cattle during the estrous cycle and during pregnancy but paid no attention to the oviducts. The extensive and critical study by Cole (7), of the mucosa with special reference to cyclic changes, covered all the different regions of the bovine genital tract except the oviducts.

It was the work of Allen (1) who first described cyclic changes in the tubes of the mouse, that focused the attention of other workers engaged in similar work in other animals, to the oviducts. In a histological description of the porcine oviduct,

published by Snyder (27) in 1923, it was shown that the successive stages of the estrous cycle were marked by clear-cut histological changes in the tubal epithelium by which the period of estrous could be distinguished from the interestrous interval. In the pig, Snyder described a high columnar epithelium with a smooth, regular surface at estrous. Anderson (2) in 1928 described the histology of the tubo uterine junction in the sow. Snyder (28) reported cyclic changes in the human tubes in which the columnar cells became tall and regular at the midmenstrual period. There were periodic changes in the fallopian tubes which involved the height of the epithelium and the morphology of the non-ciliated cells. Murphey (20) was the first worker to observe cyclic changes in the bovine oviducts in some detail. He stated that the congestion, edema and the peculiar epithelial cell desquamation was marked in the ovarian end of the oviduct of the virgin heifer from the sixteenth day onwards. Asdell (4) stated that the tubal epithelium had a distinct cycle and became lower at eight days postestrous. About this time mucous globules of cytoplasm were found which were referred to by him as secreted proteins and interpreted by others as extruded nuclei. Roark and Herman (23) concluded that the tubal cilia were longest, and the epithelium highest and smoothest during early postestrous, and coincided with the time the egg began its journey down the tube. According to Lombard et al. (17), the various regions of the bovine oviducts had distinct characteristics, primarily in the number of mucosal folds. They also studied the cellular changes in relation to the estrous cycle. Weeth and Herman (31) observed that the

cyclic changes in the oviducts during the estrous cycle indicated a high state of activity around the time of estrous followed by decreasing activity during diestrous. Epithelial-lined pockets also have been observed by these authors, though they did not appear to have any special physiological function.

Identical type of studies on the oviducts of other domestic animals have been made by other authors; the most important was the work of Cassida and McKenzie (6) who described the histology of the genital tract of the ewe during the estrous cycle. Their study mainly concerned the changes in the mucosa. Later McKenzie and Terrill (18) made further contributions to the study of the estrous, ovulation and related phenomena in the ewe. They observed in the oviducts the presence of cytoplasmic projections which resulted in cellular extrusion and stated that it was a means of cell removal which may be regarded either as a holocrine type of secretion or a process of cellular regression.

A gross description of the bovine oviduct has been reported by several investigators. Weeth and Herman (31), Arey (3), Williams (33), Roberts (24), Beaver (5), Fleming (13), Cowdry (10), Rowson (25), Sisson (26), Reynolds (22), McLeod (19), Asdell (4), Dukes (12), and Lombard et al. (17), have all contributed to the gross anatomy, a brief review of which follows.

The oviducts in the cow were elongated, tortuous, paired tubes 21 to 28 cm. long which extended from the cranial extremities of the uterine cornua to the ovaries, and served as a passageway for oocytes, fertilized ova and ascending spermatozoa.

It rested in a peritoneal fold, the mesosalphinx, which was intimately associated with the broad ligament of the uterus. The investment of the peritoneal covering of the oviduct was very close in ruminants so that the ducts were readily seen without dissection. The tubes were wiry, hard and felt nearly cartilagenous when rolled between the fingers. They were the most rigid and undilatable portion of the genital tube. The oviducts did not start immediately at the ovaries but opened up into the peritoneal cavity near the ovary where they presented a small orifice, the ostium abdomenale tubae, surrounded by fimbria. This arrangement was interesting from the fact that it gave a unique example of a breach of continuity between a gland and its excretory canal. The oviducts were the only ducts in the body which were not attached to the glands they drained, and the method by which the ovum passed across the funnel-shaped end of the tube was uncertain, though in some manner unerringly they secured the ova from the extruded follicular fluid. The smooth muscle found in the suspensory ligament of the ovary was so attached to the peritoneal wall, ovary, upper uterus and the ampulla of the tube that the contracting muscle was capable of approximating the fimbria and the surface of the ovary. It was generally believed that the fimbria came into close relationship with the ovary and may be attached to one end. When ova were discharged they fell upon the fimbria and were transferred through the lumen of the uterine tube to the uterus. It has been proved that the fimbriated anterior end of the oviduct was active during proestrous and estrous and

the musculature of the tube showed spontaneous contraction waves which varied with the stage of the estrous cycle. This was due to hormonal action, and Whitney and Burdick (32) have shown that if the oviduct was overstimulated by estrogenic substances, while the ova were traversing it, the ova may become tube locked. The ovarian end of the tube opened out and became funnel-shaped, and the mouth of the funnel was 1 cm. or more across. The junction of the oviducts with the cornua of the uterus in bovine was not abrupt since the extremities of the horns were pointed. It joined the uterine horn without any visible demarkation. The tubo-uterine junction of the cow was at the tip of the cornua. It was straight without a villus and with only a slight sphincter, which did not prevent the passage of fluid from the uterus into the tube. The tubes had their smallest diameter at the uterine end and widen into the funnel-shaped ovarian end.

Grossly, the bovine oviduct was divided into an isthmus, an ampulla and an infundibulum (Plate I, Appendix). The infundibulum also was named the pavilion or oviducal funnel. The isthmus or narrow portion of the tube joined the uterus and comprised one-third to one-half of the tubular portion of the oviduct. The fertilized ova remained in this portion three to four days, regardless of the tube length. The ampulla was the intermediate dilated portion, and the infundibulum was the funnel-shaped portion at the ovarian end of the tube with its abdominal ostium, surrounded by fimbriae. Copenhaver and Johnson (9), in the human fallopian tubes, described a fourth part after the isthmus which

they called the uterine or interstitial segment or pars uterina, which was embedded in the wall of the uterus. The infundibulum portion gradually merged into the ampulla, so that it was difficult to define the exact limits. The proportionate length of the isthmus also was quite variable. It may have comprised as little as one-third or as much as one-half of the total length of the tubular portion. All the three divisions of the duct could be differentiated histologically by the diameter, the mucosal folds and the muscular coat.

MATERIALS AND METHODS

The oviducts from mature and pregnant cows, which were normal and healthy, were collected after slaughter from the Beverly Meat Packing Company at Salina, Kansas. The material was collected within half an hour following death. Three immature oviducts were collected after necropsy from the calves available in the necropsy room. The calves died of some other diseases but the genital tracts were not affected and were in good condition without undergoing decomposition at the time of collection. Two immature oviducts and one mature oviduct were collected from embalmed calves which were used for study in the Anatomy Department. Oviducts from five animals in each class of immature and pregnant, and from six animals in the class of mature, were collected, making a total of 16 animals. The breeds represented were Jersey, Guernsey, Holstein, Ayrshire, Hereford and Hereford-Holstein cross (Table 1). The stage of pregnancy of the cow was

Table 1. The reproductive status of the oviducts at the time of slaughtering.

Serial No.	Type of oviduct	Breed	Stage when slaughtered
1	Mature	Holstein	3 days post ovulated
2	Mature	Jersey	Mid estrous period
3	Mature	Ayrshire	Mid estrous period
4	Mature	Guernsey	17 days post estrous
5	Mature-	Guernsey	Just ovulated
6	Mature	Holstein	Diestrous
7	Pregnant	Hereford	40 days pregnant
8	Pregnant	Hereford	45 days pregnant
9	Pregnant	Ayrshire	100 days pregnant
10	Pregnant	Holstein	20 days pregnant
11	Pregnant	Guernsey	65 days pregnant
12	Immature	Hereford-Holstein	9 months
13	Immature	Jersey	6 months
14	Immature	Guernsey	3 weeks
15	Immature	Guernsey	4 weeks
16	Immature	Jersey	5 months

determined from the crown-rump length of the embryo. The examination of the ovaries revealed the stages of the estrous cycle of those cows from which the mature ducts were collected. The immature oviducts were collected from calves which were three weeks to nine months old and had not attained sexual maturity.

Pieces of oviducts about 1 cm. long were taken from the ovarian extremity, the mid-region and the uterine extremity, and were placed in fixatives. For general histological observations the tissues were fixed in 10 percent neutral formalin. Other pieces of oviduct to be used for connective tissue differentiation, were fixed in Zenker's solution. Injecting 10 percent formalin through the uteroovarian artery prior to placing in formalin, fixed the tissues the best. Tissues fixed in Zenker's solution were transferred to 50 percent alcohol after 24 hours. Even if the material had been fixed in formalin, mordanting of the tissues or of the sections in Zenker's gave good results with the Crossman's modified Mallory's staining which was used for connective tissue differentiation.

The tissues were dehydrated, infiltrated and embedded in paraffin. Serial paraffin sections 6 to 8 microns thick from the anterior, middle and posterior parts of the oviduct were cut with the microtome and mounted on slides. The sections were stained with Harris hematoxylin-eosin method for general observations as this was still preferred for permanency with reasonable differentiation of nuclei and cytoplasm. Nuclei were stained blue; the cytoplasm, muscle and other structures varying shades of pink.

To bring out the collagenous tissue and reticulum in contrast with the smooth muscle, the Crossman's modification of Mallory's connective tissue staining, as described by Conn and Darrow (8), and also Heidenhain's azan triple stain were used.

A. Crossman's Modification of Mallory's Connective Tissue Stain.

Method of fixation: Zenker's or mordanting the sections in Zenker's before staining.

Staining Schedule

1. Deparaffinized and removed the xylol from the slides by passing through alcohol series.
2. Removed mercuric chloride crystals from sections by treating five minutes in Lugol's iodine solution.
3. Washed for ten minutes in running water.
4. Stained in Weigert's iron hematoxylin until nuclei were overstained. This took five minutes.
5. Washed for ten minutes in running water.
6. Stained one minute in acid fuchsin-orange G solution.
7. Rinsed in distilled water.
8. Decolorized completely in 1 percent phosphomolybdic acid using fresh solution every time.
9. Rinsed in distilled water.
10. Counterstained in aniline blue solution.
11. Rinsed in distilled water.
12. Decolorized one minute in 1 percent acetic acid.
13. Rinsed quickly in distilled water.
14. Three changes in absolute alcohol.
15. Three changes in xylol.
16. Mounted in balsam.

Collagen and reticulum took blue color, muscular tissue reddish, nuclei red and erythrocytes yellow.

B. Heidenhain's Azan Triple Stain.

This also was a modification of Mallory's triple stain in which azocarmine G replaces acid fuchsin. Many workers consider this method superior to Mallory's original combination as azocarmine G is a very precise and powerful nuclear stain which keeps well.

Method of fixation: Zenker's or formalin.

Staining Schedule

1. Took the slides through xylol and ethyl alcohol series down to water.
2. Stained in azocarmine G for eight to ten minutes at 56-60° C. in a stoppered jar.
3. Washed in distilled water.
4. Differentiated in aniline alcohol under the microscope. Cytoplasm should be light pink, and nuclei red and clear. This took about five to seven minutes.
5. Stopped differentiation by washing off aniline in 95 percent alcohol containing 1 percent glacial acetic acid for one-half minute.
6. Passed in 5 percent phosphotungstic acid until the connective tissue became completely decolorized. This took 10 to 12 minutes.
7. Rinsed rapidly in distilled water.
8. Stained in orange G-aniline blue-acetic mixture until collagenous fibers were sharply stained. This took seven to eight minutes.
9. Washed quickly in water.
10. Placed in 95 percent alcohol.
11. Passed through absolute alcohol.
12. Passed through xylol.
13. Mounted in piccolyte.

Results: Collagen and reticular connective tissue took sharp blue, muscle red to orange, chromatin of nuclei red, erythrocytes and neuroglia also red.

For mast cells, toluidine blue staining was done. This was a basic aniline dye and exhibited, in varying measure, the property of metachromasia or of staining cartilage, matrix, mucin and the granules of the mast cells a more violet or red shade than it did nuclei. This method was suitable for tissues fixed both in formalin and Zenker's. After deparaffinizing in the usual manner, the sections were stained one minute in a 0.1 percent aqueous solution of toluidine blue, washed briefly in water, dehydrated in pure acetone, cleared in xylol and mounted. If a fine reddish precipitate showed, in the case of old formalin fixed material, it was removed by rapid washing in 90 percent alcohol followed by acetone and xylol as in the original method. The nuclei took a deep blue stain, the cytoplasm a light blue, and the mast cell granules a deep violet.

All the 16 oviducts under this study were serially sectioned at the ovarian end, the mid-region and the uterine end of the ducts. It is to be stated that the changes that took place in the mature oviduct during the estrous cycles were noteworthy and were of considerable importance but that it was beyond the scope of this study to attempt to correlate the description with the different stages of the estrous cycle or with the description found in the literature. The oviducts have been collected at random and their microscopic structure described according to whether they were from a mature, immature or a pregnant animal. Animals of all ages have been included and the stage of the estrous cycle at which the mature ducts were collected was only incidental though

they did cover all the different periods of the estrous cycle.

OBSERVATIONS

The Histology of the Mature Oviduct

The wall of the oviduct consisted of three layers: the tunica mucosa, the tunica muscularis and the tunica serosa. The muscularis mucosae and the submucous coat were absent.

Tunica Mucosa. The mucous membrane was composed of a surface layer of epithelium and the underlying lamina propria. The mucous membrane was thrown into folds or plicae. The folds were running parallel to the long axis of the tube except near the ovarian end where they became irregular. The epithelium was simple ciliated columnar and was highly columnar in type in most of the area of the tube. Pseudostratified epithelium also was present and very often was noted. While the majority of the cells were ciliated, a few non-ciliated cells also were found in the epithelium. The epithelium, with its basement membrane, rested directly on the tunica muscularis as there was no submucosa. The epithelium consisted of the following three types of cells (Plate II, Appendix).

1. Ciliated columnar cells: These cells were abundant and each contained a large nucleus. The nucleus was elongated, non-vesiculated or vesiculated or was laterally compressed. The shape of the cells also varied from rectangular to pear-shaped. Goblet-shaped cells, each with a distally-situated nucleus were scattered throughout the epithelium. The arrangement of the cells also varied in different parts of the duct as in some areas the cells

were found to be closely packed together while at other areas they were loosely arranged. The cytoplasm of the cell often was granular in nature (Plate III, Appendix). The length of the cilia varied from 5 to 10 microns and exhibited prominent basal corpuscles.

2. Peg cells: Scattered among the columnar ciliated cells, slender or club-shaped, highly compressed cells were seen. They appeared as if they were flattened by the adjacent cells. The nuclei were darkly basophilic. In 1886 Frommel, as quoted by Snyder (28), distinguished in man and some of the lower animals, the non-ciliated cells and described their resemblance to secretory epithelium in contrast to the adjacent ciliated cells. Since then, periodic changes in the structure and function of these elements, chiefly with regard to ciliation, have been described in man, the pig, the rabbit and the mouse. Cowdry (11) and others have called similar types of cells in the human tubes, "peg cells," "intercalary cells" or "Stiftchenjellen." Novak and Everett (21) have stated that peg cells were wedged in between the other cells and were accepted as stages in the life cycle of the cells in the human.

3. Spherical cells: These cells were few in number and were scattered throughout the epithelium. They were small, rounded cells with a single deeply-stained, basophilic nucleus and were located close to the basement membrane. The nuclei of the spherical cells resembled the nuclei of lymphocytes and the nuclei, together with the clear zone of cytoplasm surrounding

them, made the whole spherical cell resemble a lymphocyte. Similar cells also were noted in the lamina propria. These may be wandering lymphoid cells.

Lamina Propria. The lamina propria was rich in cellular elements. The fibers were predominantly reticular though collagenous fibers were noted in the broader folds. Trautmann and Fiebiger (29) have stated that the lamina propria contained many cells, vessels and muscle fibers. This was found to agree with the observations made in this study, though very few muscle fibers were found in the lamina propria. The lamina propria contained numerous mast cells whose cytoplasmic granules stained purple with toluidine blue (Plate IV, Appendix). Lymphocytes were also found in the lamina propria. The stroma of the lamina propria was loosely arranged in the mucous membrane of the ampulla but towards the uterine end it was more compact.

The lining epithelium showed marked differences in the height of the cells, and the mucous folds showed variations in their number and formation in different regions of the oviduct.

Isthmus or Uterine End of the Duct (Plate V, Appendix). The sections were made at varying distances but not exceeding one and one-half inches from the ostium uterinum tubae. The number of folds varied from 6 to 10. The average height of the fold was 210 microns. The average width of the fold was 132 microns. The average height of the epithelium was 12 microns. The diameter of the tube at this region varied from 1 to 2 mm. The stroma was dense in this region and the folds were thicker. The folds were

comparatively low in height and the lumen was greatly reduced in diameter as compared to the rest of the tube. The folds were not seen to branch. Near the ostium the folds became thicker, due to interstitial stroma and large vessels.

Mid-region of the Duct (Plate VI, Appendix). The number of folds varied from 20 to 28. Most of them were simple, longitudinal folds. The larger folds gave off secondary folds. The average height of the fold was 750 microns. The average width was 75 microns. The diameter of the duct in this region varied from 2 to 4 mm. The connective tissue was well-developed but not as abundant as in the isthmus. Weeth and Herman (31) have stated that cilia were sparse in the mid-region, and the cilia may be better described as stereocilia or cytoplasmic projections. They have described them as occurring in tufts. In the sections that were examined during this study, the cilia were present throughout the tube and were more or less uniformly distributed.

Ovarian End of the Duct (Plate VII, Appendix). This region had a vast number of complex folds. The primary folds were high and narrow. Numerous secondary and tertiary folds also were present. The average height of the epithelium was 24 microns. The average height of the fold was 1.2 mm. The width of the fold varied from 80 to 120 microns. The primary, secondary and tertiary folds were interwoven in such a complex manner that it was not possible to take their measurements. The anastomosing of the folds divided the lumen in this region into a labyrinth of spaces as in the human duct which was described by Greep (14). More

branchings were given off by the folds near the base than at the free ends. The larger folds were reflected upon themselves, and the height of the folds increased nearer the infundibulum. In this region the stroma of the lamina propria was thin but the mucosal coat was very prominent.

Taking into consideration the oviduct as a whole, it may be said: a) the folds decreased progressively in height from the ovarian end to the uterine end of the tube; b) the stroma decreased in density from the uterine to the ovarian end of the tube; c) the diameter of the tube increased from the uterine end to the ovarian end; and d) the mucous membrane was more vascular near the ovarian end than the uterine end.

Cytoplasmic projections were observed protruding into the lumen along with extruded nuclei. This phenomenon was exhibited by some tubes in all the regions of the duct though it was more prominent in the ovarian end. Often only the nuclei were seen. Cytoplasmic projections were described by McKenzie (18) in ewes as the free ends of non-ciliated cells that extended above the level of the basal granules of the ciliated cells. Snyder (28) has stated that in the sow the non-ciliated cells were so compressed that their nuclei were forced out in the process. In this study, the cytoplasmic projections were numerous and widely distributed and it was not possible to say whether these projections were from ciliated or non-ciliated cells. Murphey (20) had referred to epithelial cells that protruded markedly above the surface of the mucosa. Such cells also have been found in few

areas in this study. Roark and Herman (23) have stated that the process of nuclear extrusion, which had a direct relation to the number and height of the cytoplasmic projections and an inverse relation to the height of the cilia and epithelium, appeared to be a process of cellular regression. Courrier and Bourg, as quoted by Roark and Herman (23), referred to nuclear extrusion as intercalary cells, and suggested that this phenomenon might in reality be a holocrine type of secretion as a means of cell removal. Asdell (4) contended that these extrusions were secreted proteins which were imbibing fluid at the periphery, thus staining less intensely in that region than in the center. The statement of McKenzie (18), that the cytoplasmic projections appeared and increased in prominence as the height of the epithelium decreased in the tubes of the ewes, holds good in the case of bovine in this study. These phenomena, described above by various authors, in the tubes of different animals as cellular desquamation, cellular regression, protein secretion or nuclear extrusion might have been due to a secretory process of the cells. According to Roark and Herman (23), more epithelial cells are involved in this phenomenon at mid-cycle than at other times.

The different tubes of the animals varied in the pseudostratification of epithelium, extrusion of nuclei, the height of the cell and to a little extent the height of the cilia. This showed clearly that the morphological changes were related to the estrous cycle.

A number of pockets of cells were observed under the epithelial lining in the various parts of the ducts (Plate VIII, Appendix). These epithelial pockets of cells resembled and gave the appearance of glands. Examination of serial cross-sections indicated that the adjacent folds of mucous membrane running in a parallel pattern close to each other gave rise to the presence of these groups of cells. The pockets had no ducts and were not true glands but were artefacts.

Tunica Muscularis. The contractions of this layer of the oviduct were undoubtedly the major propelling force of the spermatozoa. Vandermark and Moeller (30) have stated that in cows, motile and non-motile spermatozoa were found to reach the ovarian end of the oviducts within five minutes of mating. Oxytocin was found to enhance the rate of sperm transport by causing uterine and tubal contractions.

The Uterine End of the Tube. The muscular layer was thickest at this end and gradually became thinner toward the ovarian end. In the uterine portion, the musculature consisted of three layers of non-striated muscle. Next to the mucous membrane were a few longitudinal, smooth muscle fibers which were termed the inner longitudinal layer. The bulk of the muscular wall consisted of a thick band of circular fibers which constituted the middle layer. The outer longitudinal layer also was thin. A good number of elastic fibers could be seen interspersed among the muscle fibers. Near the uterine end the muscular layer averaged 400 microns in thickness. The muscular layer, which becomes thicker

and thicker near the ostium uterinum tubae, finally merged with the uterine muscles. In this area, large blood vessels were found between the circular and the outer longitudinal muscle fibers. This vascular zone resembled the stratum vasculare of the uterus.

The Mid-region. Here the muscular coat had decreased in thickness and varied from 45 to 75 microns. The inner, longitudinal layer was not discernible, and the circular layer could not be marked off distinctly from the outer, longitudinal layer. The muscular coat was interspersed with numerous elastic fibers that were more numerous than in the uterine end.

The Ovarian End. Here the muscular coat was diminished, and measured only 15 to 45 microns. It was composed chiefly of circular muscle fibers and very few isolated bundles of longitudinal fibers. The muscular coat was richly supplied with elastic tissue that formed about 50 percent of the fibers of this coat. There was no demarcation between the different layers of muscles, and longitudinal fibers could be seen extending into the serous layer.

Tunica Serosa. The serous coat was situated external to the muscular coat. It was in the form of loose connective tissue that did not closely invest the oviducts. The reflected portion of the peritoneum covered the external surface of the tunica serosa. The serous coat could be differentiated into two parts. The mesothelial part of the peritoneum was located externally, and a highly vascular layer of connective tissue, the subserosal coat, internally. The subserosal layer was rich in connective

tissue cells and contained elastic and reticular fibers in especially large numbers around the blood vessels. In the serous coat, mast cells and lymphocytes also were present.

The Histology of the Oviduct During Pregnancy

The description of the oviducts from the pregnant animals resembled, in general, that of the mature oviducts, and differed only in details. Only the differential points have been described.

Tunica Mucosa. The epithelium showed slight pseudostratification in the ovarian and mid-regions of the duct, and simple epithelium in the uterine end. The goblet cells were numerous. The cells showed marked variations in their shape, and the nucleus exhibited pyknotic changes in certain areas. Weeth and Herman (31) stated that no pronounced gestation trends were seen in the oviduct, though the cilia were sparse, poorly distributed and even non-existent in the mid-region. Troscher and Schaffer, as quoted by Snyder (28), have said that the non-ciliated cells increased in number in pregnancy in humans. They have emphasized the importance of cilia in the transport of ova through the tubes and have held that during estrous and pregnancy, as well as during menstruation in the human, the ciliated cells were diminished in number and the non-ciliated cells, which appeared to be secretory, were increased. In this study the cilia were found to be present throughout the tube and were uniformly distributed similar to that in the mature ducts. There also was no evidence of

alteration in the ratio of the ciliated to the non-ciliated cells. There was no marked change in the height of the epithelium in the three regions compared with the height of the cells in the mature ducts. The height in certain areas was comparatively less by a few microns but this was of no significance as the epithelial height of the mature oviducts varied considerably, depending upon the stage of the estrous cycle. Hence the observations of Snyder (27), that in the pregnant porcine ducts, the epithelium was more than half as high as in the non-pregnant animal, does not hold good in bovine animals. The most marked changes in the pregnant ducts were confined to the cytoplasmic projections and nuclear extrusions (Plate IX, Appendix). The cytoplasmic projections were numerous in pregnancy. Similarly, the nuclear extrusions were so numerous that in certain areas large masses of extruded nuclei were seen. Generally, the nuclear extrusion was so uniform and complete that they appeared in a continuous row of nuclei at the free ends of the cells. The cytoplasmic projections have been described by McKenzie (18) in ewes as free ends of non-ciliated cells extended above the basal granules. Similarly, the nuclei have been said to be extruded by non-ciliated cells. In the pregnant oviducts under study, these cytoplasmic projections and extruded nuclei were so much more numerous than the number of non-ciliated cells, which might be present in the tube at any time, that this statement does not hold good in bovine animals. They must have arisen from both ciliated and non-ciliated cells. All the oviducts from pregnant animals exhibited this process of

cytoplasmic and nuclear extrusions irrespective of the period of gestation, in the ovarian and mid-region of the duct. However, this process was very limited in the uterine end of the tubes.

The lamina propria was better developed, had an increased number of fibers and was highly vascular. It was infiltrated with mast cells and lymphocytes (Plate X, Appendix). The lamina propria was compact in the uterine end and was more loosely arranged in the folds of the ovarian end.

As in the case of the mature ducts, numerous epithelial pockets were seen which gave the appearance of glands, and which on examination of serial sections were found to be artefacts. Folds running close together, the junction of a primary and a secondary fold, or two small plicae joining to form one large one, on section, gave rise to the appearance of these pockets.

Tunica Muscularis. While the study of the epithelial changes in the oviducts as related to estrous cycle have been studied in different animals, no information regarding the changes in musculature was found in the available literature. As the oviducts and the uterus were closely related to each other in many respects both anatomically and physiologically, it was generally stated that the changes during the estrous cycle and pregnancy in the uterus and the ducts had a close resemblance. This statement, however, may be true to some extent, regarding the changes in the epithelium but definitely is not in regard to the changes of the muscular coat. The textbooks commonly state that the size of the uterine muscle fibers during pregnancy was about ten times the size

of the fibers from a non-pregnant uterus. If this statement was applied to the musculature of the ducts, one would also expect the muscular coat of the ducts from pregnant animals to be larger by ten times than the mature ducts. The muscular coat measured in the pregnant oviducts did not exceed 30 microns in the ovarian end and 60 microns in the mid-region. In the mature ducts the musculature showed a maximum measurement of 75 microns in the mid-region and 45 microns in the ovarian end. This showed that there were cyclic changes in the tunica muscularis of mature ducts, and that the musculature was limited to its minimum during pregnancy. As the growth cycle in the oviducts and the uterus of pregnant animals was not similar, it may also be presumed that the hormonal action which brought the changes in the two organs may be different and unrelated.

Tunica Serosa. This tunic was highly vascular and was infiltrated with numerous lymphocytes, mast cells and eosinophils.

The Histology of the Immature Oviduct

Region of the Isthmus. Tunica Mucosa. The mucous membrane was thrown into simple, small and broad leaf-like folds which varied from four to seven in number. The epithelium was simple ciliated columnar and did not show pseudostratification. The height of the epithelium was low and was reduced progressively towards the ostium uterinum tubae. Near the junction with the horn of the uterus, the epithelium was almost simple squamous in character. The folds in this area were low and the lumen was

greatly reduced in diameter and was slit-like in appearance (Plate XI, Appendix). The cilia were short and were distributed uniformly. Spherical cells, resembling lymphocytes, were present in larger numbers near the basement membrane but the slender, non-ciliated peg cells were extremely few. Goblet-shaped cells also were present in the epithelium. Cytoplasmic projections and nuclear extrusions were absent. The height of the folds varied from 75 to 150 microns. The width of the folds varied from 60 to 75 microns. The average height of the epithelium was 10 microns. The diameter of the tube varied from 600 to 700 microns.

The lamina propria contained predominantly reticular fibers. The muscle fibers from the tunica muscularis did not extend into the lamina propria. The highly cellular lamina propria was infiltrated with numerous lymphocytes and a few mast cells.

Tunica Muscularis. This consisted of an inner longitudinal, a well-developed middle circular and an outer longitudinal layer, with elastic fibers interspersed among them. The tunica muscularis varied from 150 to 200 microns in thickness. Few blood vessels were present between the circular and the outer longitudinal muscle fibers.

Tunica Serosa. This layer consisted of an outer mesothelial layer and an inner adventitial layer of connective tissue cells and elastic reticular fibers with numerous blood vessels surrounded by reticular fibers. The subserosal tissue was infiltrated with numerous lymphocytes.

Mid-region of the Oviduct. Tunica Mucosa. As in the region of the isthmus, here also the epithelium was simple ciliated columnar, though the cells were taller (Plate XII, Appendix). The number of folds varied from 12 to 16. They were simple longitudinal folds with no branchings. The cells were more closely arranged, and exhibited more peg cells than in the region of the isthmus. The cilia were present and uniformly distributed and the cells did not exhibit any cellular activity by way of cytoplasmic projections or nuclear extrusions. The lamina propria was less cellular, predominantly reticular with no muscle fibers in them. The diameter of this region was 675 microns to 1.12 mm. The average height of the fold was 310 microns. The average breadth of the fold was 45 microns. The height of the epithelium was 15 to 20 microns.

Tunica Muscularis. This was 70 microns thick and composed mainly of circular muscle fibers interspersed with a few elastic fibers. The thin, outer longitudinal layer could be marked off from the inner circular layer by the presence of blood vessels between the two layers.

Tunica Serosa. The serous coat was the same as in the region of the isthmus.

Ovarian End of the Duct. Tunica Mucosa. The mucous membrane was thrown into about 30 folds. Except at the terminal part near the ostium abdominale tubae, the folds were simple and branched (Plate XIII). The complicated pattern presented in this area by the mature ducts, where primary, secondary and tertiary folds were

interwoven, was not very typical in this region at this age. The cilia were uniformly distributed and the epithelium was mostly simple-ciliated columnar. Near the ostium the cells were closely packed, exhibited pseudostratified epithelium; there was an increase in the number of spherical and goblet cells and there was some cellular activity as shown by cytoplasmic projections. The folds were thin, composed of two layers of epithelium separated by a central layer of connective tissue containing blood vessels. The folds were interwoven. The lamina propria was highly vascular.

Tunica Muscularis. This consisted of a circular layer of 15 microns in thickness, supported by a few elastic fibers. Plexuses of blood vessels were present on either side of this coat.

Tunica Serosa. The serous coat did not exhibit any change from that of the mid-region.

The immature oviducts, considered as a whole, resembled closely the mature duct in structure but exhibited the following differences: 1) The folds or plicae were fewer and simpler in their pattern. 2) The epithelium was simple, ciliated columnar without pseudostratification and contained a large number of spherical lymphocytes. 3) The epithelial cells were loosely arranged and there was no cellular activity as would be indicated by protoplasmic protrusions or nuclear extrusions. 4) The elastic fibers in the tunica muscularis were less numerous than in the mature ducts. 5) Because the folds were few and simple,

the serial sections did not show artefacts in the form of epithelial pockets.

DISCUSSION

The basic structure of the bovine oviduct was similar to that of other animals. The present study produced the following information.

The epithelium was lined by three types of cells: ciliated columnar cells which were the majority of the cells; a few peg cells which were non-ciliated, slender and wedged in between the ciliated cells; and spherical cells resembling lymphocytes near the basement membrane.

The epithelium was more pseudostratified near the ovarian end and less near the uterine end.

The folds of mucous membrane were very few, low and simple in the isthmus; increased in number and branched in the mid-region and became numerous and increasingly complex near the ovarian end.

The lamina propria was cellular and contained predominantly reticular fibers and a few muscle fibers in addition to lymphocytes and mast cells. It decreased in density from the uterine end to the ovarian end of the tube. The lamina propria of the immature ducts did not contain muscle fibers.

The diameter of the tube increased from the uterine end to the ovarian end.

The cilia were found to be distributed uniformly throughout the duct and did not undergo any change either in the mature or

immature oviducts or during pregnancy.

Cytoplasmic protrusions and nuclear extrusions, as a result of cellular regression, were exhibited by mature and pregnant ducts. This phenomenon was more marked towards the ovarian end.

The muscular coat was interspersed with elastic fibers; was thickest in the uterine end and became progressively thinner in the mid-region and the ovarian end.

The mature ducts showed great variations in the height of the epithelium, pseudostratification, cytoplasmic protrusions and the presence of cytoplasmic granules. These changes were related to the estrous cycle.

The epithelial-lined pockets, which resembled glands, were often exhibited in the sections but were only artefacts.

In the pregnant ducts the thickness of the muscular coat and the height of the epithelium were limited to the minimum but the cytoplasmic protrusions and nuclear extrusion reached their maximum, as compared with the mature duct.

The epithelium of the immature duct was simple and did not exhibit any cellular activity. The folds were fewer and they did not form a complicated pattern.

SUMMARY

This study was undertaken as the available textbooks of histology did not contain a complete description of the minute structure of the bovine oviduct. Six mature, five from pregnant and five immature oviducts from 16 animals were studied

microscopically. The mature ducts were collected at different stages of the estrous cycle. The ducts from pregnant animals were collected during the early part of the pregnancy and immature ducts were collected from calves varying from three weeks to nine months of age. Serial sections were made from each duct at three different regions, namely the ovarian end, the mid-region and the uterine end. The sections were stained by hematoxylin and eosin and also special connective tissue stains. The three coats of the duct, the tunica mucosa, muscularis and serosa were described in detail. The distinct characteristics of various regions of the oviduct in regard to the number of mucosal folds, the pseudo-stratified condition of the epithelium, the cellular activity and the variations in the thickness of the muscular coats were noted. The differences in structure exhibited by the ducts from pregnant animals as compared to the mature ducts, which were confined mostly to cytoplasmic protrusions, were reported. The special characteristics exhibited by the immature ducts, regarding their epithelium and the simpler pattern of mucosal folds, have been described.

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APPENDIX

EXPLANATION OF PLATE I

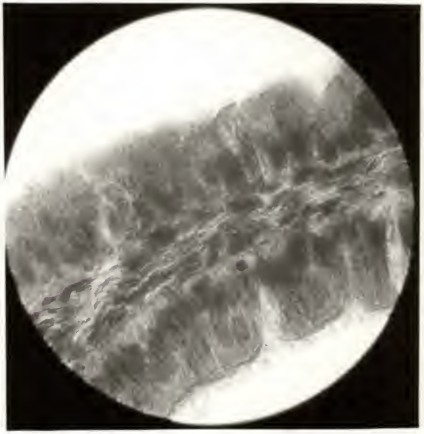
The mature bovine oviduct of the right side, showing the infundibulum, the ampulla, the isthmus and the relationship to the terminal part of the uterine horn.

PLATE I

EXPLANATION OF PLATE II

A cross-section of the mature oviduct, showing the different types of cells of the epithelium. (Harris' hematoxylin-eosin stain) 970X.

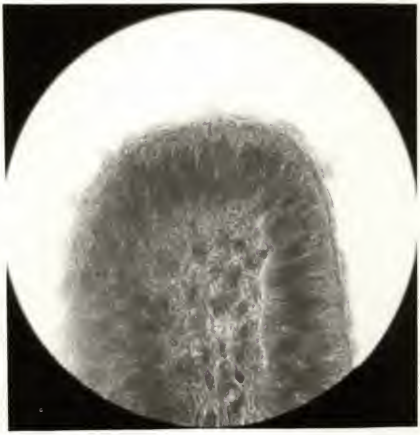
PLATE II



EXPLANATION OF PLATE III

Cross-section of the mature oviduct immediately after ovulation, showing uniformly-distributed acidophilic granules in the cytoplasm of the cells. (Harris' hematoxylin-eosin stain) 970X.

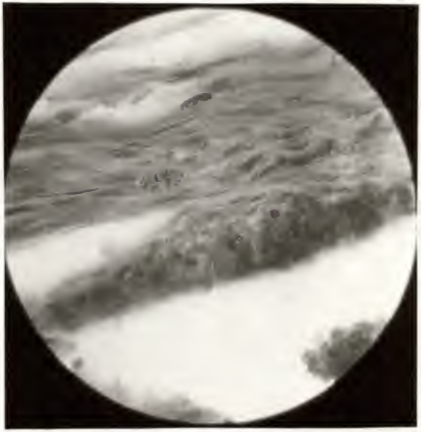
PLATE III



EXPLANATION OF PLATE IV

Cross-section of the mature oviduct, showing a mast cell in the lamina propria. (Toluidine blue stain) 970X.

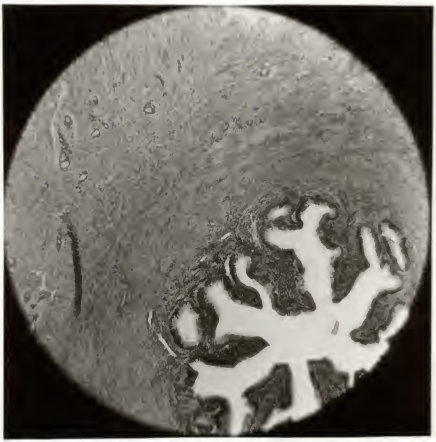
PLATE IV



EXPLANATION OF PLATE V

Cross-section through the uterine end of the mature oviduct, showing the three tunics, the simple mucosal folds and the epithelial pockets exhibited in serial sections. (Harris' hematoxylin-eosin stain) 100X.

PLATE V



EXPLANATION OF PLATE VI

Cross-section of the mid-region of the mature oviduct, showing the three tunics, an increase in the number of folds and a decrease in the thickness of the muscular coat. (Harris' hematoxylin-eosin stain) 70X.

PLATE VI

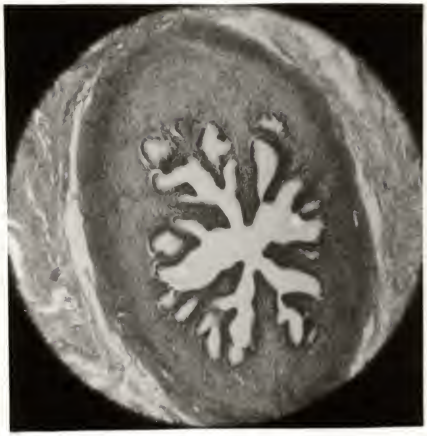
EXPLANATION OF PLATE VII

Cross-section at the ovarian end of the mature oviduct, showing the three tunics, the complex mucosal folds, a thin muscular coat and an increase in the diameter of the tube. (Harris' hematoxylin-eosin stain) 70X.

EXPLANATION OF PLATE VIII

Cross-section of the isthmus of the mature oviduct, showing the formation of artefacts which resembled glands. (Harris' hematoxylin-eosin stain) 70X.

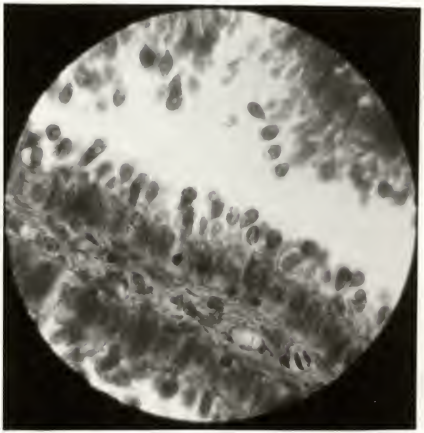
PLATE VIII



EXPLANATION OF PLATE IX

Longitudinal section of the mid-region of the oviduct from the pregnant animal, showing pronounced cytoplasmic protrusions. (Harris' hematoxylin-eosin stain) 970X.

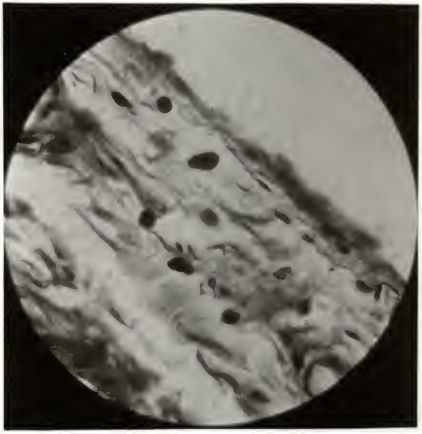
PLATE IX



EXPLANATION OF PLATE X

Cross-section of the mid-region of the oviduct from the pregnant animal, showing heavy infiltration of the lymphocytes in the lamina propria. (Toluidine blue stain) 970X.

PLATE X



EXPLANATION OF PLATE XI

Cross-section of the immature oviduct near the uterine horn, showing the low, squamous type of cells of the tunica mucosa. (Crossman's modification of Mallory's triple stain) 70X.

PLATE XI

EXPLANATION OF PLATE XII

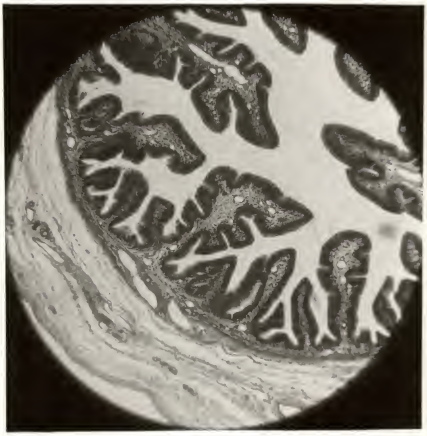
Cross-section of the immature oviduct in the mid-region, showing the three tunics, the simple folds lined by simple ciliated columnar cells. (Harris' hematoxylin-eosin stain) 70X.

PLATE XII

EXPLANATION OF PLATE XIII

Cross-section of the immature oviduct near the ovarian end, showing the three tunics and the simple branched folds without a complex interwoven pattern. (Harris' hematoxylin-eosin stain) 70X.

PLATE XIII



A HISTOLOGICAL STUDY OF THE OVIDUCT OF THE IMMATURE, MATURE AND PREGNANT BOVINE

by

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The present knowledge on the microscopic structure of the bovine oviduct was based on the descriptions of the human and other mammalian oviducts. No standard textbooks of histology contained a detailed description of the bovine oviduct. A histological study of the duct will not only help to understand clearly the physiology of reproduction but also in evaluating the pathological processes in the oviduct. To make the study of the ducts complete, the immature, the mature, and the duct during pregnancy have been included.

The oviducts were collected from six mature, five immature and five pregnant animals. Serial sections were made from each duct at three different regions, namely the ovarian end, the mid-region and the uterine end for microscopic study. The sections were stained by the Harris hematoxylin eosin method for general observations, Crossman's modification of Mallory's triple stain, and Heidenhain's azan triple stain for connective tissue differentiation and toluidine blue stain for mast cells. Photomicrographs were made to show important features.

The mature ducts were described in detail and the differences exhibited by the ducts during pregnancy and the immature ducts from the basic pattern were noted. The mature ducts consisted of a tunica mucosa, a tunica muscularis and a tunica serosa. The submucous coat and the muscularis mucosae were absent. The epithelium was simple ciliated columnar or pseudostratified ciliated columnar and consisted of three types of cells, namely the predominant ciliated columnar cells, a few peg cells and spherical cells.

The lamina propria was rich in cellular elements containing lymphocytes and mast cells and the fibers were predominantly reticular. The mucous membrane was thrown into longitudinal folds which were few and simple at the uterine end, increased in number and branched in the mid-region and became numerous and complex in nature near the ovarian end. The folds decreased progressively in height from the ovarian end to the uterine end. The stroma decreased in density; the diameter increased in caliber; and the muscular coat decreased in thickness from the uterine end to the ovarian end. The mature oviducts showed great variations in the height of the epithelium, pseudostratification, and cellular activity which were influenced by the stage of the estrous cycle, but there was no change in the cilia which were distributed uniformly in all of the ducts.

Cytoplasmic protrusions and nuclear extrusions, as a result of cellular activity, have been described and the presence of epithelial pockets, which appeared like glands but were only artefacts, have been noted.

In the ducts of the pregnant animal the muscular coat and the height of the epithelium were limited to the minimum and the cytoplasmic protrusion reached the maximum, as compared with the mature duct. The epithelium of immature ducts was simple; the folds were fewer and it did not exhibit cellular activity.